

## Supplementary Methods

### The Isle of Wight birth cohort

A whole-population birth cohort was established on the Isle of Wight (IoW) UK in 1989 to prospectively study the natural history of asthma and allergic conditions ( $n = 1,536$  subjects). After exclusion of adoptions, perinatal deaths and refusal for follow-up, 1,456 children (95%) were enrolled. The local research ethics committee approved the study and informed written parental consent was obtained for all participants at recruitment and subsequently at follow-ups, which were conducted at ages 1, 2, 4, 10, and 18 years of age. The IoW birth cohort has been described in detail elsewhere (1). In this study we utilised peripheral blood samples collected at 18 years of age from a subset of  $n = 367$  cohort participants selected for DNA methylation analysis. The selected subset are predominantly women, due to an initial research focus on future pregnancy.

### Variables

The four seasons were defined as December-February (winter), March-May (spring), June-August (summer), and September-November (autumn). Seasons of birth and seasons of blood sample collection were calculated from date of birth and date of blood sample collection respectively. For maternal socioeconomic status (SES), we utilised SES measures that do not rely on factors related to the father to reduce the number of missing values. Subjects were clustered into five levels of SES as described elsewhere (2).

Detailed questionnaires, including the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (3) were completed by parents or study subjects at each follow-up. Since the 1-year and 2-year follow-up data were collected in a relatively small time window, we combined them for analytic purposes (reported as 1-or-2 years). Total IgE was

measured in serum samples collected at age 10 and 18 years, using PRIST® (Pharmacia Diagnostics AB, Uppsala, Sweden) (4). Serum IgE data were dichotomised, with serum IgE  $\geq 200$  kU/L regarded as 'high' serum IgE. Eczema was defined as chronic or chronically relapsing, itchy dermatitis lasting more than 6 weeks with characteristic morphology and distribution, following Hanifin and Rajka criteria (5, 6). Rhinitis was defined by a problem with sneezing, or a runny or a blocked nose without a cold or the flu in the last 12 months (7). At the 1, 2, and 4 year follow-ups, the medical investigator determined the presence of asthma based on wheeze over the last 12 months and physician-diagnosed asthma. At the 10 and 18 year follow-ups asthma was defined as having "ever had asthma" and either "wheezing or whistling in the chest in the last 12 months" or "current treatment for asthma".

Estimated proportions of monocytes, B cells, natural killer (NK) cells, CD8<sup>+</sup> T cells, CD4<sup>+</sup> T cells, granulocytes and eosinophils in each blood sample were estimated from DNA methylation data using the MINFI package (8), which is based on the original code by Houseman *et al.* (9).

### **DNA extraction and arraying**

DNA from peripheral blood samples was extracted using a standard salting out procedure (10). DNA concentration was determined by Qubit quantitation. One microgram of DNA was bisulfite-converted using the EZ 96-DNA methylation kit (Zymo Research, Irvine, CA, USA), following the manufacturer's standard protocol. Epigenome-wide DNA methylation was measured using the Illumina Infinium HumanMethylation450 beadchip (450K array; Illumina, Inc., San Diego, CA, USA), which interrogates >484,000 CpG sites associated with ~24,000 genes. Arrays were processed at the Wellcome Trust Centre for Human Genetics (Oxford, UK), with multiple identical control samples assigned to each batch to assess assay variability and samples randomly distributed on arrays to control against batch effects. The proportion of methylation ( $\beta$  value) calculated for each CpG locus was determined using the

methylation module of GenomeStudio software. CpGs with one or more missing values were removed during pre-processing, and data normalisation was performed as described previously (11). Briefly, CpGs on sex chromosomes and whose probes bind to multiple sequences across the genome were removed, then the IMA R package (12) was used to pre-process data for detection  $p$  values, and normalise using peak correction and quantile normalisation. The ComBat R package (13) was then used to remove batch effects. After preprocessing and batch correction the methylation dataset for the IoW 1989 birth cohort contained 308,809 CpGs.

### **Epigenome-wide DNA methylation analyses**

The freely available R package 'ttScreening' (v1.5, available at: <http://cran.r-project.org/web/packages/ttScreening/>) (14) was used to conduct an EWAS examining the association between season of birth and CpG-specific logit-transformed DNA methylation in the Isle of Wight 1989 birth cohort at age 18 ( $n = 367$ ). The ttScreening package incorporates surrogate variable analysis (SVA) (15) as well as three options for multiple testing correction: the method controlling for false discovery rates (FDR), the Bonferroni method, and the method by use of training and testing datasets (denoted as the training-testing method below and in the manuscript).

The purpose of SVA is to produce surrogate variables for unknown or known covariates not included in the model. Details of the method for SVA can be found elsewhere (15), but the principle of this approach is to utilise the residuals obtained by regressing DNA methylation on variables of interest, such as season of birth. These residuals contain information not explained by those variables of interest. The surrogate variables are then estimated based on decomposition of the covariance matrix of the residuals (15). Incorporating the estimated surrogate variables into the regression model reduces the amount of erroneous noise and provides more accurate association estimates.

Multiple testing adjustment is with respect to the number of CpG sites to be tested in terms of their association with season of birth. The method for multiple testing correction based on controlling FDR (16) and the Bonferroni method have been applied intensively in the literature. Here we focus on a brief introduction of the method implemented in the ttScreening package. The training-testing method is based on a number of training and testing data sets. For each pair of training and testing data sets (the two data sets are mutually exclusive), the method uses the training data set to identify the associations based on linear (or robust) regressions, and then test the association in the testing data set. Regarding the selection of training and testing data sets, the subjects are randomly split into two groups such that 2/3 of the subjects are included in a training group and the remaining 1/3 are the testing group. Linear (or robust) regressions are performed across all CpGs for the training group. CpGs showing a significant association with season of birth at  $\alpha = 0.05$  will be included in a pool of candidate CpGs. These CpGs will be tested again using data in the testing group, thus providing cross-validation. The CpGs that are statistically significant again (at  $\alpha = 0.05$ ) in the testing group are deemed to be possibly informative CpGs. This process (training and testing) is repeated 100 times, and each time the training and testing sets are randomly selected. CpGs deemed as possibly informative CpGs in at least 50 out of 100 iterations are regarded as CpGs where DNA methylation is associated with season of birth. These CpGs will be further evaluated in subsequent analyses. The package tested each CpG site via cross-validations using training and testing data. We have demonstrated that the technique based on training and testing data has the potential to control both type I and type II error (14). In contrast, Bonferroni-based method lacks the ability to control type II error, while the FDR-based method cannot control type I error well (17).

Epigenome-wide analyses controlled for potential confounding factors (season of sample collection, maternal socioeconomic status (SES) and sex; see Results), and were conducted for each of the birth seasons relative to the other three combined. The package's default

parameters were used, except that the threshold number of iterations was raised from the default  $\geq 50/100$  to  $\geq 60/100$  to be more conservative. As discussed above, DNA methylation at the identified CpG sites via the triaing-testing technique is likely to be associated with season of birth after adjusting for confounding factors.

### **Gene expression**

For a subset of newborns from the IoW third generation cohort ( $n = 96$ ), RNA was extracted from cord blood samples collected into PAXgene bone marrow RNA kits. RNA quality was assessed using the Agilent 2100 BioAnalyzer system. Gene expression was measured using the Agilent SurePrint G3 Human Gene Expression 8x60k v2 microarray, with one color (Cy3) analysis including spike-in controls. Data were analysed for quality control indices using Agilent's GeneSpring software. There are two probes for the *ZFR* gene on this array: A\_24\_P311771 and A\_23\_P41818.

### **Statistical analyses**

Relative risks (RRs) of season of birth on repeated measurements of high serum IgE at ages 10 and 18, atopy at ages 4, 10 and 18, and eczema, rhinitis and asthma at ages 1-2, 4, 10 and 18, were determined using generalised estimating equations (GEEs: binomial distribution, log link function). All GEEs controlled for sex and maternal season of birth. Autumn was the reference season. Effects of cg07175945 DNA methylation ( $\beta$  values) on the expression of the *ZFR* gene (two probes) in cord blood from a subset of the IoW newborn cohort, were evaluated using generalised linear models (GLMs; normal distribution, identity link function).

To analyse whether methylation levels of the 92 season of birth-associated CpGs were also significantly associated with season of birth in an independent validation cohort, GLMs were used to compare DNA methylation (logit-transformed  $\beta$  values) between birth seasons, in 8-

year-old participants in the Dutch PIAMA cohort ( $n = 207$ ). After preprocessing, 85 of 92 season of birth-associated CpGs were retained in the PIAMA DNA methylation dataset. The PIAMA cohort is described below. GLMs (normal distribution, identity link function) for the association of season of birth with logit-transformed DNA methylation in PIAMA controlled for maternal education level (as a proxy for maternal SES), season of blood sample collection and sex – the same model used for discovery epigenome-wide analyses in the loW cohort. Autumn was the default reference season; for CpGs where autumn was the significant season of birth in the loW 1989 birth cohort, spring was used as the reference season.

To understand the functionality of the selected CpG sites, pathway analysis was performed. The gene/s annotated to each CpG were obtained from the Illumina 450K array manifest file (v1.2; available: [http://support.illumina.com/downloads/humanmethylation450\\_15017482\\_v1-2\\_product\\_files.ilmn](http://support.illumina.com/downloads/humanmethylation450_15017482_v1-2_product_files.ilmn)). Where a CpG was annotated to more than one gene, all annotated genes were included. The resulting list of season of birth-associated genes was analysed with Ingenuity pathway analysis (IPA; Qiagen). Default analysis parameters were used: the database was “Ingenuity knowledge base (genes only)”, both direct and indirect relationships were included, and the observation confidence level was set to “experimentally observed”. For IPA’s top networks analysis tool, statistical significance of biological network enrichment among the submitted list of genes is assessed using Ingenuity’s ‘score’ metric. ‘Score’ has been described previously (18) as the negative log of the  $p$  value measuring the likelihood of the input genes being found together in a network due to random chance. Scores of 2 or higher have at least a 99% confidence of not being generated by random chance; for example a score of 25 means a  $1 \times 10^{-25}$  chance that the input genes are found together in this network due to random chance (18). All IPA-identified networks were presented in the pathway results table.

Associations of DNA methylation levels (DNA methylation  $\beta$  values) of season of birth-associated CpGs at age 18 with allergic outcomes at age 18 were examined using log-linear models (binomial distribution, log link function). Allergic disease status was the outcome, and models for the effect of each CpG controlled for the same three potential confounders as the EWAS model: season of blood sample collection, maternal SES and sex.

Path analyses to identify intervening causal effects were run on CpGs associated with spring or autumn birth in the EWAS, as these were the two birth seasons involved in the significant seasonal difference in eczema risk in the loW birth cohort. Paths to allergic outcomes at age 18 years (high serum IgE, eczema, rhinitis, atopy and asthma) were therefore calculated from spring or autumn for each of these 45 CpGs using the software Mplus (v7.31).

Relationships between the variables were decomposed into direct, indirect, and total components, as described previously (19). Briefly, direct effects indicate the effect of a risk factor on an outcome that is not mediated by other variables, whereas indirect associations show the effect of a risk factor (X) on an outcome variable (Z) through an intervening variable (Y) as follows:  $X \rightarrow Y \rightarrow Z$ . The total effect of a risk factor is the combination of direct and indirect statistical relationships. In our case the indirect effect represents the potential role of DNA methylation, therefore the contribution of the indirect effect to the total is the most relevant measure. Path models used season of birth as an exposure (X), DNA methylation as a potential intermediary (Y), and allergic outcomes as the outcome (Z), as illustrated (Figure 3). Path models controlled for sex.

Batch correction entirely removes CpGs that have a missing value in any batch, which resulted in slightly different sets of CpGs in the 18-year-old data for the loW birth cohort and the newborn data for the loW third generation cohort ( $n = 175$  subjects). For analysis of season-associated methylation in newborns, pre-processed but non-batch-corrected data were used to impute missing DNA methylation values for season-associated CpGs in some of the loW third generation cohort. 78 of 92 season-associated CpGs were fully retained

after data preprocessing in the loW third generation cohort DNA methylation dataset. For the remaining 14 CpGs, loW third generation newborns in some 450K arraying batches had missing values after data preprocessing, which we imputed (cg07175945, cg24883586, cg12209881 and cg14326260: imputed in 11.9% of subjects; cg20095398, cg18803079, cg00832928, cg09781437, cg00821600 and cg18893000: imputed in 13.6% of subjects; cg24677732, cg12476443 and cg00710249: imputed in 33.5% of subjects; cg27660165: imputed in 47.2% of subjects). In total, imputed values therefore comprised 3.03% of methylation data points in newborn analyses. Imputation used the R package missForest (20). The package is built upon the random forest technique (21, 22) and applies an iterative method to predict missing values, using a random forest trained on the observed part of the dataset (20). For each of the 14 imputed CpGs, all 450K array CpGs annotated to the same gene, CpG island or intergenic gap were used as the observed data matrix.

To analyse whether methylation levels of the 92 season of birth-associated CpGs (at age 18) were already associated with season of birth at the time of birth, GLMs were used to model the effect of season of birth on logit-transformed methylation in blood samples collected at birth in loW third generation cohort newborns ( $n = 175$  subjects). The loW third generation cohort is described below. Maternal education level at 20 weeks of pregnancy (5 levels) was used as a proxy for maternal SES; season of blood sample collection was not controlled for as all newborn samples were collected at birth. 450K arraying batch was added to the model because non-batch corrected data had to be used for the imputation. In this study we utilised blood samples collected at birth from  $n = 175$  newborns: cord blood samples from  $n = 130$  subjects (born 2010 to present) and Guthrie card blood samples from  $n = 45$  subjects (born 2006-2013). GLMs (normal distribution, identity link function) for the association of season of birth with logit-transformed DNA methylation in the loW third generation cohort therefore controlled for maternal education level, sex, sample type (cord or Guthrie) and arraying batch. Autumn was the default reference season; for CpGs where

autumn was the significant season of birth in the IoW 1989 birth cohort, spring was used as the reference season.

### **PIAMA cohort**

For the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort study, pregnant women were recruited in 1996-1997 during their second trimester of pregnancy from a series of communities in the North, West, and Centre of The Netherlands. Non-allergic pregnant women were invited to participate in a “natural history” study arm. Pregnant women identified as allergic through a validated screening questionnaire were primarily allocated to an intervention arm with a random subset allocated to the natural history arm. The intervention involved the use of mite-impermeable mattress and pillow covers. The study started with 3963 participants. The PIAMA cohort has been described in detail elsewhere (23). Date of birth and sex were obtained from questionnaires completed when the child was approximately 3 months old. Maternal education was obtained from the 1-year questionnaire. Date of blood sample collection was recorded during the medical examination at age 8 years. Seasons of birth and sample collection were calculated from dates as described above. Peripheral blood samples were collected from all consenting cohort participants at age 8, and DNA from peripheral and cord blood samples was extracted using the QIAamp blood kit (Qiagen), followed by a precipitation-based concentration using GlycoBlue (Ambion). DNA concentration was determined by Nanodrop measurement and picogreen quantitation. 500 ng of DNA was bisulfite-converted using the EZ 96-DNA methylation kit following the manufacturer’s standard protocol, and DNA methylation measured using the Illumina Infinium HumanMethylation450 beadchip ( $n = 207$ ). DNA methylation data were preprocessed using the Minfi package (8), and the DASEN method from the wateRmelon package (24) was used for normalisation. The analysed subset of the PIAMA cohort had an excess of spring births ( $p = 0.021$ ,  $\chi^2$  test): winter  $n = 46$  (22.2%), spring  $n = 70$  (33.8%), summer  $n = 40$  (19.3%), and autumn  $n = 51$  (24.6%).

### **The Isle of Wight third generation cohort**

The IoW third generation cohort are the children of the IoW 1989 birth cohort. Allergy phenotype data and cord blood samples for the IoW third generation cohort are currently being collected as babies are born, and Guthrie cards have been obtained for some babies born before recruitment started. DNA was extracted from cord blood samples as described above for peripheral blood samples from the IoW birth cohort. DNA was extracted from Guthrie card blood spots using a GenSolve (Whatman) and QIAamp (Qiagen) protocol based on Beyan *et al* (25), followed by a precipitation-based concentration step. DNA methylation was measured using the Illumina Infinium HumanMethylation450 beadchip, and data pre-processing and normalisation methods were the same as for the IoW birth cohort. Sex and date of birth were recorded at birth, and season of birth was calculated as described above. Maternal education level at 20 weeks of pregnancy was obtained through questionnaire. The local research ethics committee approved the study and informed written parental consent was obtained for all participants at recruitment. Methods for 450K array processing, data preprocessing and batch correction were the same as described above for the IoW 1989 birth cohort. The seasons of birth of the IoW third generation cohort newborns were distributed equally across the four seasons ( $p = 0.925$ ,  $\chi^2$  test): winter  $n = 46$  (26.3%), spring  $n = 46$  (26.3%), summer  $n = 41$  (23.4%), and autumn  $n = 42$  (24.0%).

Statistical analyses were conducted using SPSS software (v22.0, IBM).

## Supplementary References

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**Supplementary Table 1: Comparison of the subset of loW 1989 birth cohort subjects in which DNA methylation was profiled, to the whole cohort**

Factor	DNA methylation subset	Whole cohort	<i>p</i> value
Season of birth			0.288
Winter	122 (33.2%)	499 (32.5%)	
Spring	84 (22.9%)	364 (23.7%)	
Summer	71 (19.3%)	353 (23.0%)	
Autumn	90 (24.5%)	320 (20.8%)	
Season of sample collection			0.807
Winter	95 (26.0%)	144 (24.2%)	
Spring	89 (24.4%)	142 (23.9%)	
Summer	61 (16.7%)	113 (19.0%)	
Autumn	120 (32.9%)	195 (32.8%)	
Maternal socioeconomic status cluster			0.902
1 (lowest)	58 (16.2%)	209 (15.4%)	
2	60 (16.8%)	240 (17.7%)	
3	103 (28.9%)	403 (29.7%)	
4	103 (28.9%)	394 (29.0%)	
5 (highest)	33 (9.2%)	111 (8.2%)	
Sex			<0.0001
Male	122 (33.2%)	786 (51.2%)	
Female	245 (66.8%)	750 (48.8%)	
High serum IgE (≥200 kU/L)			0.460
No	103 (71.0%)	309 (67.8%)	
Yes	42 (29.0%)	147 (32.2%)	
Eczema			0.759
No	302 (82.3%)	1068 (81.6%)	
Yes	65 (17.7%)	241 (18.4%)	
Rhinitis			0.428
No	244 (66.5%)	841 (64.2%)	
Yes	123 (33.5%)	468 (35.8%)	
Atopy			0.025
No	237 (65.5%)	500 (58.6%)	
Yes	125 (34.5%)	353 (41.4%)	
Asthma			0.085
No	316 (86.1%)	1074 (82.3%)	
Yes	51 (13.9%)	231 (17.7%)	

Numbers of subjects in each subgroup are shown (with % of total); *p* values were obtained

using  $\chi^2$  tests. The full cohort has *n* = 1,536 subjects recruited at birth and *n* = 1,456

available for follow-up; where numbers total to less than this the difference is due to missing

data. High serum IgE, eczema, rhinitis, atopy and asthma status were assessed at age 18.

**Supplementary Table 2: Comparison of potential confounding factors across seasons of birth**

Factor	Season of birth				p value
	Winter	Spring	Summer	Autumn	
Season of blood sample collection					<b>&lt;0.0001</b>
Winter	14 (11.5%)	15 (18.3%)	32 (45.1%)	34 (37.8%)	
Spring	48 (39.3%)	2 (2.4%)	5 (7.0%)	34 (37.8%)	
Summer	29 (23.8%)	13 (15.9%)	11 (15.5%)	8 (8.9%)	
Autumn	31 (25.4%)	52 (63.4%)	23 (32.4%)	14 (15.6%)	
Maternal SES cluster					<b>0.030</b>
1 (lowest)	19 (16.1%)	13 (15.5%)	12 (17.1%)	14 (16.5%)	
2	23 (19.5%)	12 (14.3%)	11 (15.7%)	14 (16.5%)	
3	24 (20.3%)	33 (39.3%)	28 (40.0%)	18 (21.2%)	
4	41 (34.7%)	15 (17.9%)	15 (21.4%)	32 (37.6%)	
5 (highest)	11 (9.3%)	11 (13.1%)	4 (5.7%)	7 (8.2%)	
Sex					0.942
Male	40 (32.8%)	28 (33.3%)	22 (31.0%)	32 (35.6%)	
Female	82 (67.2%)	56 (66.7%)	49 (69.0%)	58 (64.4%)	
Height at 18 (cm)	169.6 (8.9)	168.7 (8.6)	168.4 (9.3)	169.0 (9.1)	0.813
Cell type proportions					
CD8 <sup>+</sup> T cells	6.2% (4.4%)	7.5% (5.4%)	7.2% (7.5%)	7.1% (4.4%)	0.097
CD4 <sup>+</sup> T cells	12.7% (4.6%)	12.7% (5.0%)	11.5% (4.4%)	12.4% (4.1%)	0.272
NK cells	9.0% (6.3%)	9.7% (5.4%)	8.8% (6.3%)	8.7% (6.2%)	0.360
B cells	4.6% (2.5%)	4.6% (2.3%)	4.5% (2.5%)	4.9% (2.4%)	0.792
Monocytes	8.0% (2.6%)	7.9% (2.1%)	7.7% (2.6%)	7.7% (2.5%)	0.920
Granulocytes	56.4% (9.5%)	54.2% (10.9%)	56.8% (11.4%)	55.9% (9.0%)	0.334
Eosinophils	2.2% (2.2%)	2.5% (2.9%)	2.8% (3.4%)	2.4% (2.8%)	0.848

This analysis examined the subset of IoW birth cohort participants with DNA methylation data at age 18 ( $n = 367$ ). For categorical variables the numbers of participants (and percentage within each season of birth) are shown; for continuous variables the average (and standard deviation) within each season of birth are shown. Where numbers total to less than 367, this is due to missing data. SES = socioeconomic status.

Supplementary Table 3: List of 92 CpGs significantly associated with season of birth

CpG ID	Season of birth	Coeff.	Iter. (TT)	P value	High serum IgE	Eczema	Rhinitis	Atopy	Asthma	Ave. $\beta$	$\beta$ diff.	PIAMA dir.	CHR	MAPINFO (v37)	Annotated gene/s	CpG location relative to gene	CpG island
cg07175945	Autumn	0.1186	90	5.05E-08	0.932	0.207	0.785	0.587	0.389	0.2388	0.0154	1	5	32444062	ZFR	Body	Island
cg25719132	Autumn	0.1443	77	3.82E-07	0.389	0.274	0.853	0.169	0.711	0.0467	0.0054	1	2	70520671	SNRPG	Body	Island
cg20095398	Spring	-0.1307	77	8.48E-07	0.166	0.191	0.922	0.845	0.742	0.6179	-0.0132	1	4	1571649			S_Shore
cg25382472	Autumn	0.1386	75	9.57E-07	0.547	0.977	0.779	0.412	0.460	0.0726	0.0064	1	1	47779932	STIL	TSS200	Island
cg24883586	Winter	-0.1752	74	1.02E-06	-	0.372	0.898	0.749	0.434	0.1632	-0.0137	1	8	37757094	RAB11FIP1	TSS200	Island
cg06365303	Winter	0.1926	79	1.06E-06	0.466	<b>0.028</b>	0.465	0.451	0.716	0.2294	0.0177	1	20	2801844			Island
cg14136781	Winter	-0.2031	76	1.10E-06	0.635	0.445	0.505	0.539	0.746	0.9041	-0.0088	1	9	124087974	GSN	Body	
cg08089851	Summer	0.1320	86	1.10E-06	0.544	0.762	0.227	0.842	0.976	0.0486	0.0037	1	3	12598413	MKRN2	TSS200	Island
cg10063512	Autumn	0.0950	70	1.93E-06	0.600	0.948	0.490	0.284	0.664	0.1010	0.0081	1	8	37594142	ERLIN2;ERLIN2;ERLIN2	TSS200;1stExon;5'UTR	Island
cg12209881	Spring	-0.6048	80	1.98E-06	-	0.589	0.072	<b>0.009</b>	0.208	0.9432	-0.0485	1	17	53339697			N_Shelf
cg02294108	Summer	-0.1979	69	3.86E-06	0.590	0.605	0.055	0.465	0.532	0.0280	-0.0023	0	9	115480060	C9orf80	5'UTR	N_Shore
cg06577708	Summer	0.1131	70	4.40E-06	0.139	0.813	0.942	0.494	0.541	0.1257	0.0069	0	3	64008893	PSMD6	Body	Island
cg25730142	Autumn	0.1108	73	4.51E-06	0.775	0.468	0.935	0.783	0.701	0.1375	0.0086	0	20	32031702	SNTA1	TSS200	Island
cg22062239	Autumn	0.1222	73	5.10E-06	0.524	<b>0.039</b>	0.804	0.339	0.117	0.0288	0.0017	1	16	31106011	VKORC1	1stExon	Island
cg24677732	Autumn	-0.1812	70	5.30E-06	0.071	0.075	0.269	0.441	0.329	0.0331	-0.0043	1	10	6019526	IL15RA;IL15RA	1stExon;5'UTR	Island
cg08962590	Winter	-0.0896	66	5.69E-06	0.452	0.595	0.095	0.567	0.704	0.0936	-0.0033	1	3	180631158	FXR1;FXR1	5'UTR;Body	S_Shore
cg24799448	Summer	0.1357	70	5.77E-06	0.858	0.602	-	-	0.094	0.0328	0.0028	1	11	1404215			Island
cg23922755	Autumn	-0.0932	64	5.95E-06	0.731	0.164	0.147	0.989	0.241	0.8668	-0.0061	0	4	3443656	HGFAC	TSS200	
cg05137146	Spring	-0.1265	69	5.97E-06	0.582	0.971	0.840	0.847	0.871	0.0657	-0.0071	1	16	30103511	TBX6	TSS1500	Island
cg16286281	Summer	-0.2566	78	6.05E-06	-	0.290	0.739	0.518	0.363	0.9309	-0.0115	0	8	119120052	EXT1	Body	N_Shelf
cg10208132	Winter	0.1205	69	6.13E-06	0.385	0.497	0.515	0.176	0.289	0.9168	0.0040	-	4	16362584			Island
cg00941797	Winter	-0.0978	71	6.42E-06	0.855	0.474	0.551	0.281	<b>0.049</b>	0.0411	-0.0022	0	14	81407711			Island
cg01390039	Summer	0.1606	67	6.55E-06	0.082	0.468	0.870	0.862	<b>0.022</b>	0.9440	0.0064	0	16	89315293			Island
cg00787537	Spring	-0.1390	68	6.77E-06	0.155	0.807	0.943	0.711	0.890	0.1037	-0.0151	0	1	211307839	KCNH1	TSS1500	S_Shore
cg20440552	Winter	0.1599	71	7.02E-06	0.344	0.060	0.665	0.779	0.497	0.9605	0.0039	1	6	44025753			
cg18803079	Autumn	-0.1020	75	8.08E-06	0.203	0.292	0.425	0.360	0.650	0.6213	-0.0077	-	1	64014643	EFCAB7;DLEU2L	Body;TSS200	
cg27660165	Winter	0.1047	60	8.21E-06	0.484	0.864	0.616	0.387	0.154	0.0492	0.0023	1	1	156784036	SH2D2A	Body	Island
cg24577417	Spring	0.1195	70	8.54E-06	-	0.420	0.155	<b>0.003</b>	0.575	0.8953	0.0044	1	6	168378559	HGC6.3	TSS1500	N_Shore
cg15670990	Autumn	0.1048	67	9.15E-06	0.292	0.368	<b>0.007</b>	0.239	0.944	0.0484	0.0031	1	6	32016115	TNXB	Body	
cg03987115	Autumn	0.1015	67	9.26E-06	0.914	0.859	0.281	0.705	0.904	0.0438	0.0029	1	7	27212870	HOXA10	Body	Island
cg14029372	Autumn	0.1276	68	9.71E-06	-	0.453	0.058	<b>0.045</b>	0.096	0.0844	0.0056	0	12	95867830	METAP2;METAP2	1stExon;5'UTR	Island
cg02370858	Summer	-0.1205	63	1.12E-05	0.590	0.902	0.114	<b>0.039</b>	<b>0.016</b>	0.0426	-0.0027	0	2	74700039	CCDC142;MRPL53	3'UTR;TSS200	S_Shore
cg12476443	Winter	0.1563	75	1.14E-05	0.629	0.572	0.159	0.473	0.456	0.9538	0.0043	1	2	26624228	C2orf39	TSS1500	N_Shore
cg07903677	Summer	0.1944	63	1.17E-05	0.440	0.284	0.262	0.108	0.343	0.4434	0.0266	0	1	111218079	KCNA3	TSS1500	S_Shore
cg16166262	Autumn	0.0839	64	1.19E-05	0.251	0.496	0.572	0.132	0.346	0.5641	0.0188	-	22	43037594	CYB5R3;CYB5R3;ATP5L2	5'UTR;Body;TSS1500	
cg16121765	Spring	0.3226	70	1.26E-05	0.363	0.226	0.813	0.285	0.948	0.7627	0.0369	1	10	123442007			
cg19728226	Autumn	-0.1114	62	1.28E-05	0.682	0.479	0.847	0.153	0.661	0.0913	-0.0062	0	2	176968744			N_Shore
cg11592497	Winter	0.1609	66	1.48E-05	0.389	<b>0.039</b>	0.792	0.576	0.989	0.1876	0.0128	1	17	40273580	KAT2A;HSPB9	TSS200;TSS1500	N_Shore
cg10273890	Summer	0.1750	66	1.55E-05	0.516	0.153	0.780	0.164	0.200	0.9039	0.0116	1	7	155024732			
cg18462141	Autumn	-0.1132	63	1.71E-05	0.198	0.502	0.297	0.746	0.526	0.6544	-0.0136	-	11	49872414			Island

Supplementary Table 3: List of 92 CpGs significantly associated with season of birth

CpG ID	Season of birth	Coeff.	Iter. (TT)	P value	High serum IgE	Eczema	Rhinitis	Atopy	Asthma	Ave. $\beta$	$\beta$ diff.	PIAMA dir.	CHR	MAPINFO (v37)	Annotated gene/s	CpG location relative to gene	CpG island
cg22784836	Winter	0.1174	62	1.80E-05	0.812	0.351	0.451	0.375	0.173	0.7935	0.0089	1	11	1219516			N_Shelf
cg00832928	Autumn	-0.0941	67	1.84E-05	-	0.293	0.311	0.316	0.929	0.6733	-0.0037	1	3	150329458	<i>SELT</i>	Body	Island
cg18388519	Spring	-0.1496	61	1.93E-05	0.718	0.816	0.287	<b>0.026</b>	0.176	0.9576	-0.0032	0	13	44543930			Island
cg12124731	Summer	0.1806	66	1.98E-05	0.522	0.066	<b>0.046</b>	0.242	0.058	0.0405	0.0050	1	1	151170706	<i>PIP5K1A</i>	TSS1500	N_Shore
cg02054964	Autumn	0.0919	66	1.99E-05	0.196	0.605	0.599	0.702	0.499	0.8759	0.0096	1	7	157643770	<i>PTPRN2</i>	Body	
cg03008165	Autumn	-0.0980	61	2.02E-05	-	0.208	0.285	0.516	0.676	0.8237	-0.0065	-	11	77024667			
cg11155697	Autumn	0.1907	66	2.11E-05	-	<b>0.046</b>	0.899	0.643	0.202	0.0141	0.0016	1	17	46671234	<i>LOC404266;HOXB5</i>	Body;TSS200	Island
cg02127589	Spring	0.1452	60	2.32E-05	0.531	0.220	0.519	0.210	<b>0.047</b>	0.0853	0.0098	-	8	12458309			N_Shore
cg05274741	Autumn	0.0850	63	2.36E-05	0.629	0.339	0.646	0.585	0.157	0.5326	0.0098	0	3	20053940	<i>C3orf48</i>	TSS200	
cg09726703	Summer	-0.1464	61	2.40E-05	0.401	<b>0.017</b>	0.110	0.535	0.418	0.9507	-0.0038	1	2	236441769	<i>AGAP1</i>	Body	N_Shelf
cg25841675	Spring	-0.1696	62	2.49E-05	0.806	0.259	0.670	0.753	0.545	0.0504	-0.0041	1	7	148822694	<i>ZNF425;ZNF398</i>	Body;TSS1500	N_Shore
cg08134053	Winter	-0.0950	60	2.55E-05	0.535	0.257	0.555	0.964	0.459	0.0437	-0.0016	1	2	24232856	<i>MFSD2B</i>	TSS200	Island
cg22630160	Summer	-0.1546	60	2.55E-05	0.614	0.746	0.462	0.962	0.531	0.0558	-0.0031	-	6	26033475	<i>HIST1H3B;HIST1H2AB</i>	TSS1500;1stExon	Island
cg07490151	Autumn	0.0818	64	2.57E-05	0.724	0.751	0.240	0.333	0.794	0.1937	0.0107	1	6	143772072	<i>PEX3;PEX3;ADAT2</i>	5'UTR;1stExon;TSS1500	Island
cg14124066	Spring	0.1380	70	2.60E-05	0.448	0.612	0.476	0.538	0.546	0.9210	0.0039	0	9	125015960	<i>RBM18</i>	Body	
cg12199165	Autumn	0.0838	62	2.63E-05	0.167	0.952	0.482	0.353	0.501	0.2242	0.0118	1	10	103577593	<i>MGEA5</i>	1stExon	Island
cg04210284	Summer	0.2150	61	2.69E-05	0.102	0.373	0.312	0.388	0.618	0.0287	0.0035	1	5	1445561	<i>SLC6A3</i>	TSS200	Island
cg06886896	Winter	-0.1143	69	2.76E-05	0.811	0.237	0.452	0.356	0.797	0.0634	-0.0037	0	2	120436636	<i>TMEM177</i>	TSS200	Island
cg22584335	Autumn	0.1150	60	3.11E-05	0.310	0.947	0.771	0.945	0.897	0.1292	0.0072	1	2	27806067	<i>ZNF512</i>	Body	Island
cg12799739	Winter	0.0980	60	3.15E-05	0.929	0.092	0.246	0.459	0.433	0.9141	0.0043	1	3	97595056	<i>CRYBG3</i>	TSS1500	
cg07905273	Winter	-0.0842	64	3.19E-05	0.739	0.898	0.456	0.094	0.980	0.0530	-0.0021	0	12	45610446	<i>ANO6;PLEKHA9</i>	Body;TSS1500	Island
cg01592526	Summer	-0.1408	63	3.32E-05	0.803	0.215	0.167	0.875	0.595	0.0346	-0.0028	1	17	73663357	<i>RECQL5;SAP30BP</i>	TSS200;TSS200	Island
cg04658543	Winter	0.2641	61	3.46E-05	0.919	0.482	0.185	-	0.497	0.0601	0.0084	1	12	113904848	<i>LHX5</i>	Body	Island
cg03545635	Winter	-0.1144	60	3.50E-05	-	0.932	-	0.828	-	0.8997	-0.0059	0	7	2471551	<i>CHST12</i>	5'UTR	N_Shore
cg13772515	Summer	-0.2410	64	3.52E-05	0.671	0.637	0.062	0.842	0.239	0.4611	-0.0277	1	2	115409517	<i>DPP10</i>	Body	
cg09781437	Autumn	-0.2273	64	3.67E-05	0.456	0.096	0.804	0.204	0.125	0.0223	-0.0031	1	11	2923410	<i>SLC22A18AS;SLC22A18;SLC22A18</i>	5'UTR;5'UTR;TSS200	Island
cg05710512	Autumn	0.1089	62	3.83E-05	0.379	0.468	0.838	0.714	0.617	0.9185	0.0028	1	5	32501420			
cg00009944	Summer	-0.1420	62	3.87E-05	0.894	0.320	0.773	0.530	0.583	0.9536	-0.0034	0	21	48017232			N_Shore
cg08905496	Summer	-0.1545	63	4.10E-05	0.150	0.701	0.905	0.929	0.970	0.0486	-0.0043	0	1	165414332	<i>RXRG;RXRG</i>	5'UTR;1stExon	
cg00821600	Autumn	-0.2135	64	4.12E-05	0.310	0.256	0.601	0.309	0.209	0.6565	-0.0383	1	2	142889384	<i>LRP1B</i>	TSS200	S_Shore
cg17389677	Spring	0.1452	62	4.15E-05	-	0.700	<b>0.019</b>	0.543	0.760	0.0362	0.0027	0	3	44518859	<i>ZNF445</i>	5'UTR	Island
cg04096150	Summer	-0.1255	61	4.27E-05	-	0.230	0.207	0.160	0.153	0.8941	-0.0078	0	15	40098188	<i>GPR176</i>	Body	
cg18893000	Winter	0.2144	60	4.43E-05	-	0.182	0.339	0.388	0.687	0.8261	0.0212	0	7	107931294	<i>NRCAM</i>	5'UTR	
cg01519350	Summer	-0.1549	63	4.45E-05	0.881	0.482	0.657	0.942	0.822	0.0571	-0.0038	1	3	137906342	<i>ARMC8;ARMC8</i>	5'UTR;1stExon	Island
cg02341139	Spring	-0.3300	63	4.72E-05	0.159	0.936	0.431	0.439	0.805	0.9495	-0.0098	0	2	113015451			S_Shelf
cg08244518	Winter	-0.1790	60	4.96E-05	0.632	0.712	0.773	0.391	0.612	0.8975	-0.0108	1	22	41684706			S_Shelf
cg14326260	Winter	0.1329	62	5.12E-05	0.979	0.543	0.425	0.259	0.074	0.9416	0.0042	1	20	3913389	<i>RNF24</i>	3'UTR	
cg18254123	Autumn	-0.2402	64	5.53E-05	0.158	0.977	0.177	0.243	0.101	0.2015	-0.0215	0	15	80544661			Island
cg00710249	Winter	-0.1355	60	6.72E-05	-	0.193	0.898	0.175	0.846	0.9714	-0.0022	1	16	2144849	<i>PKD1</i>	Body	S_Shelf

Supplementary Table 3: List of 92 CpGs significantly associated with season of birth

CpG ID	Season of birth	Coeff.	Iter. (TT)	P value	High serum IgE	Eczema	Rhinitis	Atopy	Asthma	Ave. $\beta$	$\beta$ diff.	PIAMA dir.	CHR	MAPINFO (v37)	Annotated gene/s	CpG location relative to gene	CpG island
cg14794041	Winter	-0.1301	61	6.75E-05	0.461	0.988	0.317	0.874	0.668	0.4081	-0.0129	0	19	6768159	<i>SH2D3A</i>	TSS1500	Island
cg15661753	Winter	0.1080	60	6.96E-05	0.975	0.336	0.964	0.438	0.973	0.2969	0.0143	0	12	49627010			N_Shore
cg09304653	Autumn	0.1129	64	7.32E-05	0.604	0.778	0.369	0.405	0.290	0.0728	0.0046	1	7	141251275	<i>AGK</i>	5'UTR	Island
cg00946960	Winter	-0.1778	62	7.35E-05	-	0.135	<b>0.046</b>	0.443	0.955	0.8082	-0.0155	0	10	14470897			
cg17679246	Spring	-0.1605	66	7.89E-05	0.817	0.507	0.070	0.280	0.837	0.0349	-0.0038	1	2	173420265	<i>PDK1</i>	TSS1500	Island
cg01040890	Autumn	-0.0843	60	8.42E-05	0.643	0.180	0.305	0.811	0.631	0.7956	-0.0084	1	5	180073780	<i>FLT4</i>	Body	N_Shore
cg02049232	Summer	-0.1282	64	8.83E-05	0.918	<b>0.045</b>	0.198	0.857	0.419	0.1623	-0.0101	1	8	145556129	<i>SCRT1</i>	3'UTR	Island
cg26355472	Summer	-0.1452	69	9.35E-05	0.192	0.642	0.509	0.582	0.810	0.0315	-0.0027	1	1	47134199	<i>ATPAF1</i>	TSS200	Island
cg25952663	Autumn	-0.1558	61	9.38E-05	0.500	0.096	0.888	0.601	0.716	0.9152	-0.0070	1	12	54450826	<i>FLJ12825</i>	TSS1500	N_Shelf
cg09704553	Autumn	0.0760	60	1.01E-04	0.199	0.170	0.199	0.061	0.124	0.3062	0.0149	1	12	27167052	<i>TM7SF3</i>	1stExon	Island
cg18965620	Autumn	-0.1123	60	1.14E-04	0.766	0.345	0.583	0.549	<b>0.011</b>	0.9062	-0.0013	0	1	180144025	<i>QSOX1</i>	Body	
cg19379625	Summer	-0.1012	62	1.27E-04	0.624	0.987	0.579	0.873	0.710	0.0450	-0.0028	1	4	71859087	<i>DCK</i>	TSS200	N_Shore
cg10542584	Winter	-0.0918	60	1.71E-04	0.768	0.116	0.340	<b>0.008</b>	0.801	0.1184	-0.0034	0	3	62356223	<i>FEZF2</i>	Body	Island

Columns: CpG ID, the significantly associated season of birth; regression coefficient, number of iterations selected (out of 100) and p value for the association with the significantly associated season of birth (from training-testing); associations with allergic outcomes at age 18 (significant p values are highlighted in bold font); average methylation level ( $\beta$  value); difference between average  $\beta$  value for the significant season of birth and average of the other three; whether the direction of effect was validated in the PIAMA cohort (0=no, 1=yes, -=CpG not available in PIAMA dataset); the chromosome and location of the CpG (in v37 of the human genome); the gene/s annotated to the CpG, location relative to annotated genes, and location relative to CpG islands (extracted from the Illumina 450K array manifest file).

**Supplementary Table 4: Association of cg07175945 with *ZFR* gene expression**

<i>ZFR</i> probe	Probe location		cg07175945 association	
	Genomic coordinates	Relative to gene	<i>p</i> value	Coeff.
<b>A_24_P311771</b>	<b>32385686-32385627</b>	<b>Exon 15/20 (coding)</b>	<b>0.040</b>	<b>-2.816</b>
A_23_P41818	32354917-32354858	Exon 20/20 (3'UTR)	0.726	-0.470

Effects of cg07175945-*ZFR* methylation on *ZFR* gene expression were analysed in the subset of the loW newborn cohort for which both cord blood DNA methylation and gene expression data were available ( $n = 93$ ). Probe coordinates on chromosome 5 (genome v37) were obtained from the array manifest. UTR = untranslated region. *P* values and regression coefficients from GLMs are shown. Significant probes are shown in bold font.

**Supplementary Table 5: Genetic networks containing genes differentially methylated by season of birth.** Ingenuity pathway analysis (IPA)'s networks tool found twelve networks containing differentially methylated genes, including three statistically significant networks each containing more than 10 genes.

Molecule names in bold font are those detected in the EWAS as differentially methylated.

Molecules in network	Count	Score	Top diseases and functions
ACTB, Actin, <b>ADAT2</b> , AGTR1, ATM, <b>CYB5R3</b> , <b>EFCAB7</b> , <b>GSN</b> , <b>HGFAC</b> , Histone H3, HNF1A, HOXB3, <b>HOXB5</b> , Hsp90, <b>IL15RA</b> , KAT5, KRAS, <b>LHX5</b> , <b>LRP1B</b> , MAPK9, MET, <b>METAP2</b> , NCL, NR3C1, PCNA, POU5F1, PTEN, <b>RECQL5</b> , <b>RXRG</b> , <b>SAP30BP</b> , <b>SCRT1</b> , SRC, SVIL, TLR1, <b>VKORC1</b> , <b>AGK</b> , ATF3, CHEK1, CSF2, CTNNB1, CXCL12, ELAVL1, ERK1/2, ESR1, <b>EXT1</b> , <b>FLT4</b> , <b>GPR176</b> , GSK3B, <b>HOXA10</b> , Hsp90, <b>INIP</b> , INTS3, ITGA5, ITGB1, ITGB3, <b>KCNA3</b> , KRAS, LSS, MET, NABP1, <b>NRCAM</b> , <b>PKD1</b> , <b>PSMD6</b> , <b>QSOX1</b> , <b>RAB11FIP1</b> , <b>SH2D2A</b> , <b>SLC6A3</b> , TNF, TP53, YWHAG	15	27	Embryonic development, organismal development, cancer
ABLIM1, ACTB, ALDOC, CDCA4, E2F1, EGFR, ELAVL1, ELF1, <b>FXR1</b> , G6PD, GNE, <b>HIST1H2AB</b> , <b>HIST1H3B</b> , Hsp90, IGF2BP3, <b>KAT2A</b> , KDM5B, <b>MGEA5</b> , NUPR1, <b>PDK1</b> , <b>PIP5K1A</b> , PRKCA, PRKCD, <b>PTPRN2</b> , RB1, S100A2, <b>SLC22A18AS</b> , <b>SNRPG</b> , <b>STIL</b> , TP73, TUBA1A, TUBA4A, TUT1, UBAP2L, <b>ZNF512</b>	14	24	Cell cycle, cellular movement, cell death and survival
CHST12, STOX1	12	20	Cell death and survival, cell cycle, cancer
KIAA1524, <b>SLC22A18</b>	1	2	Cardiovascular disease, organismal injury and abnormalities, reproductive system disease
<b>ARMC8</b> , miR-30a-3p (and other miRNAs with seed UUUCAGU)	1	2	Cancer, gastrointestinal disease, organismal injury and abnormalities
SECISBP2, <b>SELT</b>	1	2	Hereditary disorder, skeletal and muscular disorders, developmental disorder
DRAP1, <b>SH2D3A</b>	1	2	Metabolic disease, amino acid metabolism, protein synthesis
<b>KCNH1</b> , TLR7	1	2	Embryonic development, organismal development, tissue development
SBDS, <b>TNXB</b>	1	2	Cell-to-cell signalling and interaction, cellular growth and proliferation, endocrine system development and function
<b>PEX3</b> , PEX19	1	2	Developmental disorder, endocrine system disorders, gastrointestinal disease
ABCA1, ADRA1D, <b>SNTA1</b>	1	2	Cellular assembly and organisation, cellular function and maintenance, hepatocellular peroxisome proliferation
	1	2	Lipid metabolism, molecular transport, small molecule biochemistry

Count = the number of genes containing season-associated methylated within each network; Score = an Ingenuity-derived statistical significance metric.